ARTICLES

Sequential pathology of the gills of Coho salmon with a combined diatom and microsporidian gill infection

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Abstract

A large group of 40 gram Coho salmon smolts experienced 60% mortality within the first week after introduction to cages in sea-water. Histological and ultrastructural examination of sequential samples of gill tissue revealed a dramatic suppurative branchitis accompanied by extensive fusion of gill lamellae. This dramatic host response and subsequent high mortality appeared to be in response to a bloom of *Corethron*-like diatoms. A further complication was an extensive infection of endothelial cells of the gill vasculature by a microsporidian protozoan.

Résumé

Pathologie séquentielle des branchies de saumon coho présentant une infection combinée par des diatomées et des microsporidies

Un taux de mortalité de 60 % fut observé, chez un grand nombre de jeunes saumons coho de 40 g, et ce, durant la première semaine après leur introduction dans des réservoirs d'eau de mer. Les examens histologiques et la microscopie électronique séquentielle d'échantillons et tissus des branchies démontrèrent une branchite suppurative marquée, accompagnée de fusions extensives des lamelles des branchies. Cette réponse marquée de l'hôte et le taux de mortalité élevé semblaient être causés par une prolifération de diatomées *Corethron*-like. Cette observation était compliquée par une infection extensive des cellules endothéliales de la vascularisation des branchies par un protozoaire microsporidien.

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Introduction

The clinical and economic impact of blooms of diatoms and other algae is often rapid and catastrophic in both finfish and shellfish culture. Numerous pathogenic algae have been identified, and those pertinent to British Columbia salmon culture have been partially reviewed (1). In general, algal blooms can adversely affect fish through the action of elaborated toxins, metabolic waste products, and their physiological consumption of oxygen (2). They can also act as mechanical irritants of gills. An example of this latter mechanism is the damage caused by the frustule and spiny processes of the diatom *Chaetoceros convolutus* (3).

Sporozoan protozoa of the phylum Microspora are common parasites of many families of fish (4), although their role as pathogens of salmonids is more limited. Infection of the gills by a microsporidian pathogen (*Loma* sp.) has been described affecting gills of Chinook salmon (*Oncorhynchus tshawytscha*), the key features being thrombosis and vasculitis associated with xenoparasitic cysts of the cell-hypertrophy tumor type affecting endothelial cells (5).

In this report we detail the results of a clinical and pathological investigation of a fish kill attributable principally to an algal diatom bloom affecting the gills of commercial marine salmon from the coast of British Columbia. A microsporidian resembling *Loma* sp. was also associated with gill lesions.

Clinical findings and processing of samples

This disease outbreak affected a previously healthy group of 100,000, 40 g Coho salmon (*O. kisutch*). Losses in these salmon smolts began in early October 1987, shortly after their transfer to salt water net-pens located along the coast of British Columbia, and 60% died within the first week.

Some affected smolts retained parr marks. Clinically, the fish hung listlessly near the top and sides of the net pen. Rapid, labored respiration with constant opercular flaring was typical. Some fish had petechial hemorrhages mid-way along fin rays. Oxygen levels in the water were normal and ranged from 9-11 mg/L. The cage operators noticed increased turbidity of the water and Secchi disk turbidity readings measured only

2 m during the first week in which the fish were clinically affected. A bloom of the diatom *C. convulutus* was suspected, based on past blooms of this particular diatom in this area at similar times during previous years. Based on a reduction in turbidity, and an improvement in Secchi disk readings to over 3 m, the diatom bloom was considered to have abated after the first week.

Nevertheless, mortality continued through November and early December of 1987, with approximately 10% of the remaining stock dying each week. Surviving fish had extremely poor growth rates, and many subsequently became affected by bacterial kidney disease (caused by *Renibacterium salmoninarum*).

Tissue specimens from moribund and clinically affected fish were collected at the following intervals after initial mortalities occurred: one week (15 fish), two weeks (20 fish), one month (20 fish), and two months (15 fish). Samples were preserved in Bouin's fixative, transferred to 70% alcohol and routinely processed for histology. Sections were stained with hematoxylin and eosin, toluidine blue, periodic acid-Schiff, methenamine silver, Masson's trichrome, Giemsa, and Gram stains. Samples for scanning electron microscopy were obtained by de-embedding tissues from paraffin blocks. These were then postfixed in 1% osmium tetroxide, dehydrated in ascending concentrations of alcohol, dried in a critical point drier and coated with palladium in a sputter coater prior to examination.

Histopathological and ultrastructural findings

The gills of each of the 15 fish collected one week after losses began had dramatic histological lesions. Fusion of adjacent lamellae was extensive. Large numbers of necrotic lamellar (respiratory) epithelial cells were exfoliating into interlamellar spaces, accompanied by low to massive numbers of mature, well-lobulated neutrophils. Spongiosis of filamental (nonrespiratory) epithelium was marked and extensive. Neutrophils were present in extracellular spaces between the double-layers of lamellar epithelium, between filamental epithelial cells, and in lymphatic-like spaces draining toward the central venous sinus of the filament. Associated with areas of most severe lesions, and distributed in lesser numbers over the entire gill, were diatoms (Figure 1). They were present impacted amongst the gill rakers of the gill arch, between filaments, and frequently trapped between adjacent lamellae.

The nucleus and chloroplasts of the diatoms were clearly distinguishable with hematoxylin and eosin. Features of the frustule, which are important in diatom identification, were more easily seen with toluidine blue (Figure 2). The cell envelope was cylindrical with convex valves present on either end of the cylinder. Long setae, laden with small spines, emerged as a corona from around the cell envelope. The diatoms appeared singly in all sections.

The apparent fate of entrapped diatoms was followed through sequential sampling. In fish collected during the first week, diatoms were present in interlamellar

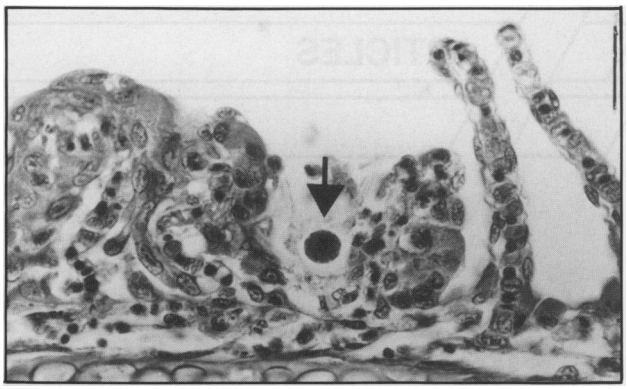

Figure 1. Diatom cell (arrow) between gill lamellae. Hematoxylin and eosin. Bar = 25 μm.

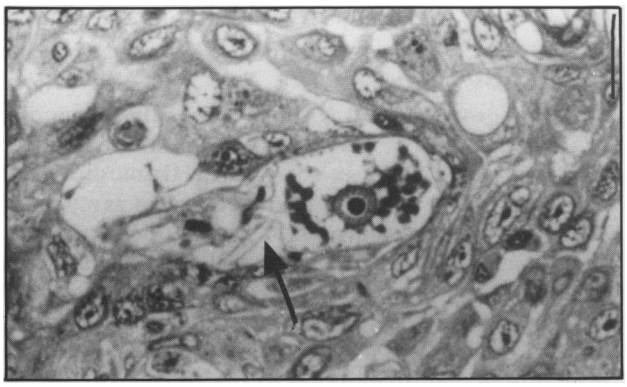

Figure 2. Diatom cell embedded in gill epithelium. The frustule has numerous setae (arrow) emerging from it. Toluidine blue. Bar = 10 μm.

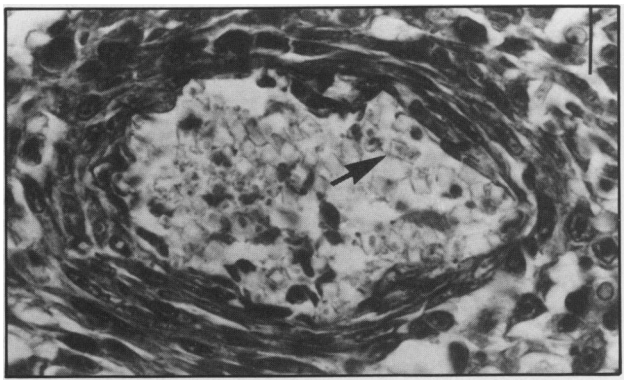

Figure 3. Multilayered wall of lamellar epithelial cells surrounding diatom setae. Setae are rectangular (arrow) in cross-section. Hematoxylin and eosin. Bar = 8 μm.

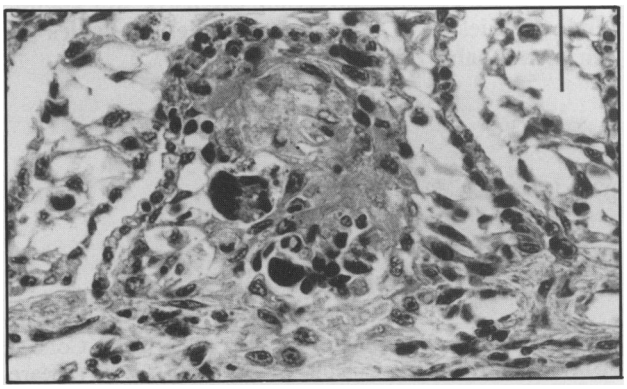

Figure 4. Multinucleated giant cell in inflamed gill tissue. Hematoxylin and eosin. Bar = 33 μm.

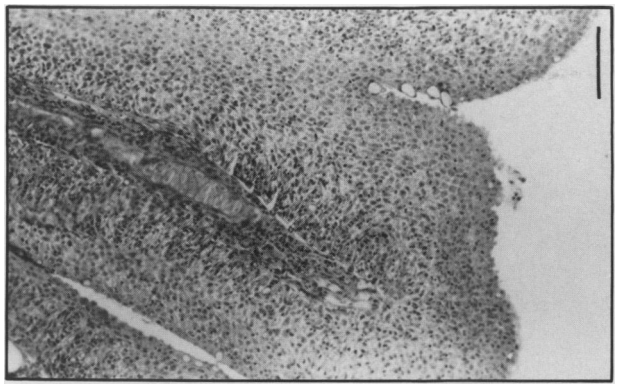

Figure 5. Proliferative nodule of epithelial tissue at the distal end of a filament. Hematoxylin and eosin. Bar = 100 μm.

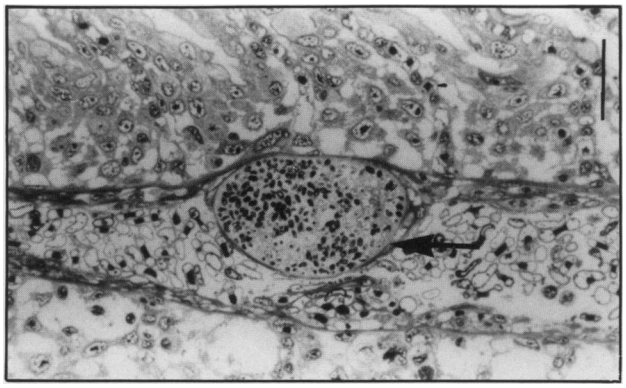

Figure 6. Microsporidial cyst (arrow) containing spores. Toluidine blue. Bar = 38 μm.

spaces, the long setae effectively bracing the diatom cells between adjacent lamellae. Diatoms were also trapped between fused lamellae. Setae did not penetrate lamellar or filamental epithelial tissue, although they did occasionally penetrate arch epithelium. Gill tissues collected from each of the 20 fish at two weeks contained diatoms surrounded by multi-layered walls of lamellar epithelial cells (Figure 3). Multinucleated giant cells (Figure 4) were also present in this wall and these sometimes contained remnants of setae which in cross-section had a diamond or rectangular shape. With the exception of occasional giant cells, the inflammatory pattern during the second week did not differ much from that of the first week.

Sections of gill tissue from each of 20 fish collected one month after the initial losses, seemed to have fewer diatoms, many of which had undergone partial degeneration of their frustule and internal cellular components. The exudate, although still neutrophil-rich, now contained greater numbers of lymphocytes and macrophages. Remnants of some diatom frustules were found in filamental tissues and some had reached the central venous sinus of the filament. (This sinus is a low-flow, low-hematocrit sinusoid situated centrally along the length of each gill filament). The internalization of diatoms was accompanied by a shift in the focus of the inflammatory response toward these central venous sinuses and mild fibroplasia within the sinusoids with production of both type 1 (Masson's trichrome positive) and type 3 (methenamine silver positive) collagen. Fibroplasia was still not evident in the tissue wall that surrounded those diatoms trapped between lamellae, nor was it present in the proliferative filament epithelium.

A dramatic feature was the chronic proliferative response of the lamellar and filamental epithelial tissues. Within two weeks of the initial exposure to the diatoms, proliferation of an acanthotic epithelium several layers thick and devoid of mucous cells obscured interlamellar spaces of the gills of all fish examined. The proliferative response at this time occurred in a multifocal random fashion along the length of the filament. In contrast, gills of all 20 fish collected one month after the initial losses, had massive nodules of spongiotic hypertrophic epithelium generally restricted to the tips of filaments (Figure 5).

Histological examination of gill tissue also revealed multifocal intracellular cysts of a microsporidian parasite. These were present in the gills of each fish examined at each collection period. The cysts were located in the endothelial cells of the gill vasculature or in the endothelial-like pillar cells of the lamellae (Figure 6). The cysts measured up to 130 μm in diameter. Within the cysts were uninuclear meronts and maturing oval spores (2×4 μm). The spores had enlarged anterior polaroplasts and coiled polar filaments. Cysts in pillar cells occasionally were associated with telangiectatic pillar cell channels. Cysts within the central venous sinuses of the filaments frequently were surrounded by a maturing connective tissue coat and an accumulation of neutrophils and lymphocytes. Cysts were also present in the endothelium of afferent and efferent branchial arteries. In some cases, however, the cysts were located perivascularly, and in these cases there was a leukocytoclastic vasculitis, with margination of neutrophils and fibrinoid necrosis of the tunica media.

Other tissues were normal except for the renal interstitium. In both anterior and posterior kidney of each of ten fish examined one month after the initial losses, there was a marked increase in myeloid precursors of granulocytes with an associated decrease of mature granulocytes.

On ultrastructural examination of the gills, the cylindrical cell envelope of the diatom exhibited several intercalary lines indicative of cell growth along the pervalvar axis (Figure 7). The valves were convex, and setae arose as a double corona from the entire circumference of the girdle which encircled one end of the cell envelope. Setae did not penetrate host tissue, but frequently were trapped between fused lamellae (Figure 8).

Hyperplastic regions of gills were characterized by variably sized masses of swollen epithelial cells. The epithelial cells retained their roughly polygonal shape but bulged markedly from their attachment sites to adjacent epithelial cells. Deep plasma membrane ruffles replaced the characteristic microridged pattern of these cells. Multi-focal fusion of adjacent filaments occurred on the gills of 12 to 20 fish examined one month after initial losses. Points of fusion were generally restricted to the distal tips of filaments, specifically those areas with pronounced hyperplasia.

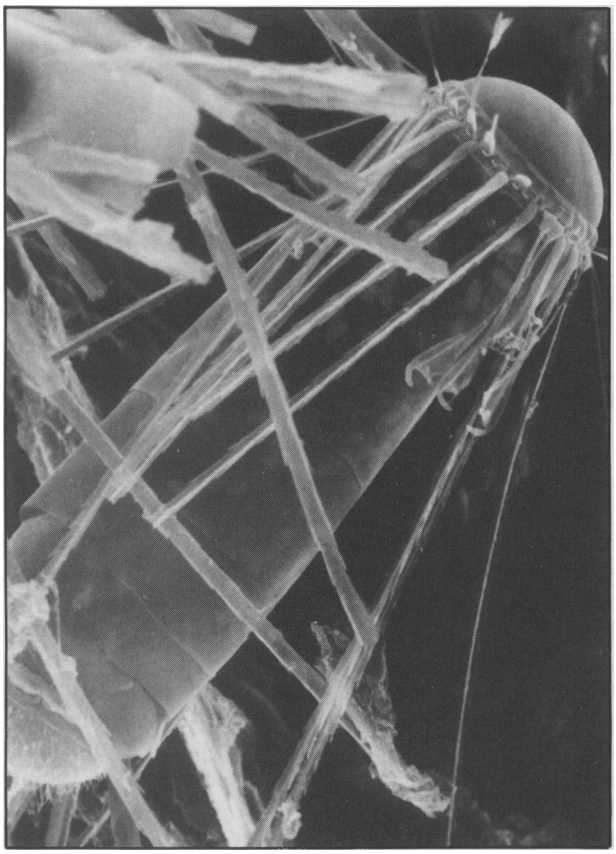

Figure 7. Scanning electron micrograph of diatom. Setae arise as a double corona from one end of the cell envelope.

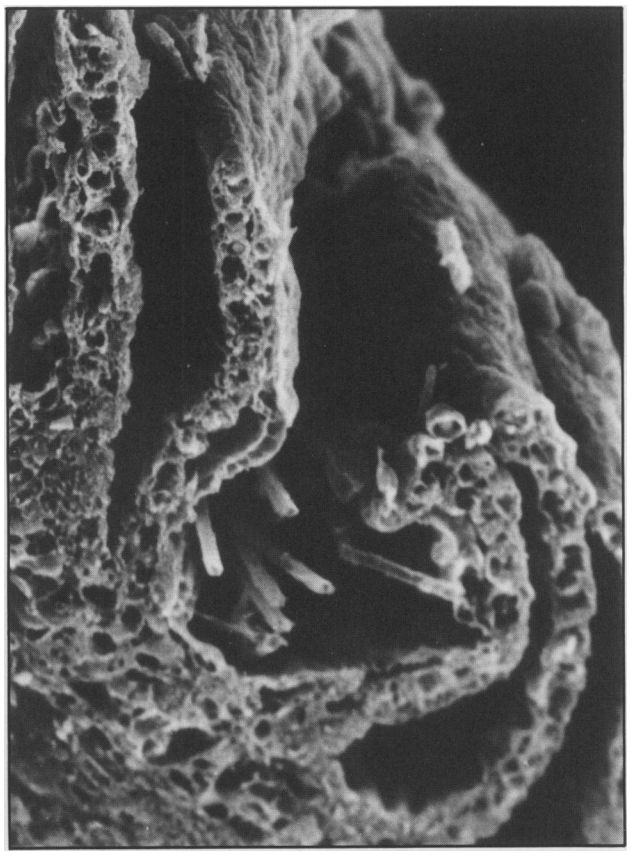

Figure 8. Scanning electron micrograph of sectioned gill tissue. Lamellae are curled over impacted diatom setae.

Discussion

Based on the size of its spores, its vascular orientation, and the nature of the host response, the microsporidian pathogen most closely resembles the descriptions of *Loma* sp., a known respiratory pathogen of salmonids (5). It is likely that the parasite infected these fish in fresh water, since cysts with mature spores were already present in fish less than one week after transfer to seawater. Vasculitis and thrombus formation in larger afferent and efferent branchial arteries may have created circulatory abnormalities. This could reduce arterio-arterial lamellar perfusion and gas exchange, or could affect the arteriovenous circulation and nutritive supply to gill tissue by blocking nutritive vessels which emanate from efferent filament arteries (6,7).

Despite the presence of the microsporidian, mortalities were considered to be more likely the result of the severe host response mounted toward the diatom, compounded by the osmotic stress of transfer to seawater. The diatom bloom may have been a discrete event, possibly even only one week in duration. This was suggested by the cage-operator's report of reduced turbidity of the water. In addition, the apparent number of diatoms in the gills of fish collected during the first week, was greater than in the gills of fish collected during subsequent weeks. The actual duration and population kinetics of this diatom bloom, are however, unknown.

From the noncolonial nature of the diatom, features of the frustule, and the circumferential corona of nonbranching spiny setae, this diatom resembles members of the genus *Corethron* (Family Corethronaceae) (8). This family and the large Chaetoceraceae family, are within the same sub-order Coscinodiscineae of the Bacillariales order of diatoms (8). The role of a similar diatom, *Chaetoceros convolutus* in causing gill lesions has been previously described (1,3). The jagged ends of fractured hollow setae of *Chaetoceros* penetrate gill epithelium and cause gill damage and hemorrhage. By contrast, the setae of *Corethron* did not penetrate host epithelial tissues, except for occasional nonrespiratory areas on the arch. Instead, the impacted diatom apparently became enveloped by the process of lamellar fusion. In chronic cases, the shift in the inflammatory response to a more central position in the filament, specifically the lymphatics and extracellular tissues adjacent to the central venous sinus, may reflect internalization of foreign diatom material. Alternatively, the inflammatory response may in part have been directed toward the microsporidia encysted in the central venous sinus.

Pathophysiological events that led to high mortality in this case may have included the following. First, extensive lamellar fusion reduces the surface area for oxygen and electrolyte exchange; this process is a common, nonspecific event in gill diseases of many etiologies (9,10,11). Lamellar fusion also inhibits the essential intermeshing of lamellae of adjacent filaments. This would have a major role in reducing the resistance which water encounters as it passes through the gill, leading to an anatomical water shunt, allowing water to pass through the gill sieve without coming in

close contact with blood (6). This, compounded with the rapid rate of respiration observed clinically, would yield a ventilation-perfusion mismatch, which would have a negative effect on respiratory efficiency. Second, the neutrophil-rich response was dramatic, and the release of neutrophil secretions would probably have had a deleterious effect on the host epithelial tissue. Third, the chronicity of the inflammatory response, and the ongoing necrosis and exfoliation of epithelial cells associated with the persistence of the diatom frustule were probably important factors initiating the severe proliferative response of filamental and epithelial tissues. Epithelial hyperplasia is a common response in gills to injury (9,10,11); a major consequence of this is an increased diffusion distance for gas transfer (6). Fourth, the gill has a major role in maintaining electrolyte and osmotic balance after smolts are transferred to sea-water from fresh-water hatcheries (7). Gill damage during this critical adjustment period may therefore be particularly deleterious.

The morphological pattern of gill lesions associated with this diatom was distinctive in that acute generalized suppurative branchitis is an unusual response of gill tissue. The multinucleated giant cells detected in tissues surrounding the diatom were also an unusual feature (giant cells are less common in fish than in other vertebrates) and suggest a foreign-body type of response, presumably directed towards the persistent silica of the entrapped diatom frustule. Similar responses to entrapped and persistent foreign material are observed in pulmonary silicosis in laboratory mammals (12). The characteristic acute neutrophil-rich inflammatory response, seen in experimental pulmonary silicosis is currently thought to be mediated by chemotaxins released from alveolar macrophages during phagocytosis of silica particles (13). An analogous macrophage population has not, however, been identified for the gill lamellae. Monitoring of the diatom content of sea-water and an ability to move net-pens to sites where the number of pathogenic diatoms is acceptable, are essential elements in minimizing losses due to diatom blooms. A presumptive clinical diagnosis of gill disease due to diatoms can be easily verified with histological or whole mount examination of gill tissue from diseased fish.

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